



Europäisches Patentamt
European Patent Office
Office européen des brevets

⑪ Publication number:

0018072
A1

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EUROPEAN PATENT APPLICATION

㉑ Application number: 80300700.4

㉓ Int. Cl.: C 07 C 103/52, A 61 K 37/02

㉒ Date of filing: 06.03.80

㉔ Priority: 30.03.79 US 25552
30.03.79 US 25533
30.03.79 US 25534
30.03.79 US 25532
30.03.79 US 25531
30.03.79 US 25555
30.03.79 US 25535
30.03.79 US 25554
30.03.79 US 25553
17.10.79 US 85824

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㉖ Date of publication of application: 29.10.80
Bulletin 80/22

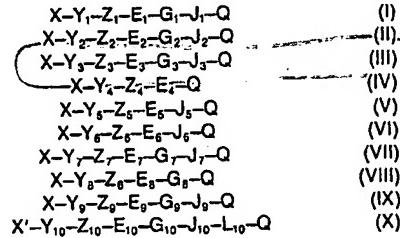
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㉘ Designated Contracting States: BE CH DE FR GB IT SE

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㉚ Synthetic peptides having pituitary growth hormone releasing activity.

㉛ Novel peptides having the following amino acid sequence



E_i is selected from a group consisting of glycine, alanine, valine, leucine, isoleucine, proline, hydroxyproline, serine, threonine, cysteine, methionine, aspartic acid, glutamic acid, asparagine, glutamine, and histidine;

E_j is selected from a group consisting of glycine, alanine, valine, leucine, methionine, and isoleucine;

J_i is selected from a group consisting of glycine, alanine, D-alanine, valine, D-valine, leucine, D-leucine, isoleucine, D-isoleucine, proline, D-proline, hydroxyproline, D-hydroxyproline, serine, D-serine, threonine, D-threonine, cysteine, D-cysteine, methionine, and D-methionine;

Y_i is selected from a group consisting of tyrosine, D-tyrosine, tryptophan, D-tryptophan, phenylalanine, and D-phenylalanine;

G_i is selected from a group consisting of lysine and arginine;

Y_j is selected from a group consisting of D-lysine and D-arginine;

J_j is selected from a group consisting of glycine, alanine, valine, leucine, isoleucine, proline, hydroxyproline, serine, threonine, cysteine, methionine, aspartic acid, glutamic acid, asparagine, glutamine, arginine, and lysine;

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and the pharmaceutically acceptable salts thereof, wherein X is selected from a group consisting of -NH₂, -NHCH₃, and -N(CH₃)₂;

Y₁, G₁, Y₂, G₂, E₄, Z₅, J₅, Y₆, G₆, Z₇, G₇, Y₈, G₈, Y₉, Y₁₀ and G₁₀ are selected from a group consisting of tyrosine, tryptophan, and phenylalanine;

Z₁, J₁, Z₂, Z₃, E₃, Y₄, Z₄, E₅, G₅, E₆, J₆, Y₇, E₇, Z₈, E₈, E₉, E₁₀, Z₁₀, and J₁₀ are selected from a group consisting of D-tyrosine, D-tryptophan, and D-phenylalanine;

J₃ and Z₆ are selected from a group consisting of glycine, alanine, valine, leucine, isoleucine; proline, hydroxyproline, serine, threonine, cysteine, and methionine;

J_9 is selected from a group consisting of natural amino acids and the D-configuration thereof;

X' is selected from a group consisting of $-NHCOC_2$, $-NH_2$, $-NHCH_3$, and $-N(CH_3)_2$;

E_{10} is selected from a group consisting of glycine, alanine, valine, leucine, isoleucine, serine, threonine, methionine, asparagine, and glutamine;

L_{10} is selected from a group consisting of asparagine, glutamine, glutamic acid, arginine, lysine, serine, and threonine; and

Q is a C-terminal functional group selected from a group consisting of amide ($-CONH_2$), amide lower alkyl ($-NHR$), amide di(lower alkyl) ($-CONR_1R_2$), lower alkoxy ($-CH_2OR$), hydroxy ($-CH_2OH$), carboxy ($-COOH$) and the lower ester derivatives thereof ($-COOR$).

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SYNTHETIC PEPTIDES HAVING
PITUITARY GROWTH HORMONE RELEASING ACTIVITY

Background of the Invention

1. Field of the Invention

This invention relates to peptides which possess pituitary growth hormone releasing activity.

5 2. Description of the Prior Art

Growth hormone, which is secreted from the pituitary, causes growth of all tissues of the body that are capable of growing. In addition, growth hormone is known to have the following basic effects on 10 the metabolic process of the body:

1. Increased rate of protein synthesis in all cells of the body;

2. Decreased rate of carbohydrate utilization in cells of the body;

15 3. Increased mobilization of free fatty acids and use of fatty acids for energy.

A deficiency in growth hormone secretion can result in various medical disorders, such as some instances of dwarfism.

20 Various ways are known to release growth hormone. For example, chemicals such as arginine, L-dihydroxyphenylamine (L-DOPA), glucagon, vasopressin, and insulin induced hypoglycemia, as well as activities such as sleep and exercise, indirectly cause growth 25 hormone to be released from the pituitary by acting in some fashion on the hypothalamus perhaps either to decrease somatostatin secretion or to increase an unknown endogenous growth hormone-releasing hormone or both.

30 Compounds which directly act on the pituitary to release growth hormone include prostaglandin E₁ and E₂, theophylline, and cyclic nucleotides. However, these compounds neither specifically release growth

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hormone nor are they believed to act at the putative growth hormone-releasing hormone receptors in the peripheral membrane of the pituitary cell to initiate growth hormone release.

- 5 In addition, under special conditions certain chemically defined peptides, e.g., vasopressin, thyroid-releasing hormone (TRH), luteinizing hormone-releasing hormone (LH-RH), α -melanocyte-stimulating hormone (α -MSH), glucagon, substance P, neurotensin;
- 10 Met-enkephelin, α -endorphin, cholera-enterotoxin, and basic myelin protein, act to release growth hormone from the pituitary. However, only TRH acts directly on the pituitary to elicit this response. Furthermore, the above listed peptides release other pituitary
- 15 hormones and under most experimental conditions do not release growth hormone. For example, TRH does not release growth hormone in normal rats or in normal humans or from pituitaries of normal rats or monkeys. In vitro, TRH releases growth hormone, prolactin, and
- 20 thyroid stimulating hormone (TSH) and in vivo TRH releases these hormones from bovine pituitary.

Vasopressin's induced release of growth hormone is considered to be due to a non-specific response to stress caused by administration of high dosages of vasopressin.

Accordingly it would be highly desirable to have a compound which directly acts on the pituitary under normal experimental conditions to effect the release of growth hormone therefrom. Such peptides

30 would be useful in vitro as unique research tools for understanding how growth hormone secretion is regulated at the pituitary level and would also be useful in vivo to treat symptoms related to growth hormone deficiencies.

35

Summary of the Invention

In accordance with the present invention

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there is provided peptides which act directly on the pituitary under normal experimental conditions in vitro to release growth hormone therefrom.

These growth hormone releasing peptides can 5 be utilized in vitro as unique research tools for understanding, inter alia, how growth hormone secretion is regulated at the pituitary level.

Also, the growth hormone releasing peptides of the instant invention can also be administered in 10 vivo to increase growth hormone release.

More particularly, this invention encompasses novel peptides having a formula selected from a group consisting of

- | | | |
|----|--|---------|
| 15 | X-Y ₁ -Z ₁ -E ₁ -G ₁ -J ₁ -Q | (I) |
| | X-Y ₂ -Z ₂ -E ₂ -G ₂ -J ₂ -Q | (II) |
| | X-Y ₃ -Z ₃ -E ₃ -G ₃ -J ₃ -Q | (III) ✓ |
| | X-Y ₄ -Z ₄ -E ₄ -Q | (IV) |
| | X-Y ₅ -Z ₅ -E ₅ -J ₅ -Q | (V) |
| | X-Y ₆ -Z ₆ -E ₆ -J ₆ -Q | (VI) |
| 20 | X-Y ₇ -Z ₇ -E ₇ -G ₇ -J ₇ -Q | (VII) |
| | X-Y ₈ -Z ₈ -E ₈ -G ₈ -Q | (VIII) |
| | X-Y ₉ -Z ₉ -E ₉ -G ₉ -J ₉ -Q | (IX) |
| | X'-Y ₁₀ -Z ₁₀ -E ₁₀ -G ₁₀ -J ₁₀ -L ₁₀ -Q | (X) |

and the pharmaceutically acceptable salts thereof,
25 wherein

X is selected from a group consisting of
-NH₂, -NHCH₃, and -N(CH₃)₂;

Y₁, G₁, Y₂, G₂, E₄, Z₅, J₅, Y₆, G₆, Z₇,
G₇, Y₈, G₈, Y₉, G₉, Y₁₀, and G₁₀ are selected from a
30 group consisting of tyrosine, tryptophan, and phenylalanine;

Z₁, J₁, Z₂, Z₃, E₃, Y₄, Z₄, E₅, G₅, E₆,
J₆, Y₇, E₇, Z₈, E₈, Z₉, E₉, Z₁₀, and J₁₀ are selected
from a group consisting of D-tyrosine, D-trypophan,
35 and D-phenylalanine;

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J_3 and Z_6 are selected from a group consisting of glycine, alanine, valine, leucine, isoleucine, proline, hydroxyproline, serine, threonine, cysteine, and methionine;

5 E_1 is selected from a group consisting of glycine, alanine, valine, leucine, isoleucine, proline, hydroxyproline, serine, threonine, cysteine, methionine, aspartic acid, glutamic acid, asparagine, glutamine, and histidine;

10 E_2 is selected from a group consisting of glycine, alanine, valine, leucine, methionine, and isoleucine;

15 J_2 is selected from a group consisting of glycine, alanine, D-alanine, valine, D-valine, leucine, D-leucine, isoleucine, D-isoleucine, proline, D-proline, hydroxyproline, D-hydroxyproline, serine, D-serine, threonine, D-threonine, cysteine, D-cysteine, methionine, and D-methionine;

20 Y_3 is selected from a group consisting of tyrosine, D-tyrosine, tryptophan, D-tryptophan, phenylalanine, and D-phenylalanine;

G_3 is selected from a group consisting of lysine and arginine;

25 Y_5 is selected from a group consisting of D-lysine and D-arginine;

30 J_7 is selected from a group consisting of glycine, alanine, valine, leucine, isoleucine, proline, hydroxyproline, serine, threonine, cysteine, methionine, aspartic acid, glutamic acid, asparagine, glutamine, arginine, and lysine;

J_9 is selected from a group consisting of natural amino acids and the D-configuration thereof;

X' is selected from a group consisting of $-\text{NHCHOCH}_3$, $-\text{NH}_2$, $-\text{NHCH}_3$, and $-\text{N}(\text{CH}_3)_2$;

35 E_{10} is selected from a group consisting of glycine, alanine, valine, leucine, isoleucine,

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serine, threonine, methionine, asparagine, and glutamine;

L_{10} is selected from a group consisting of asparagine, glutamine, glutamic acid, arginine, 5 lysine, serine, and threonine; and

Q is a C-terminal functional group selected from a group consisting of amide ($-\text{CONH}_2$), amide lower alkyl ($-\text{NHR}$), amide (di(lower alkyl) ($-\text{CONR}_1\text{R}_2$), lower alkoxy ($-\text{CH}_2\text{OR}$), hydroxy ($-\text{CH}_2\text{OH}$), 10 carboxy ($-\text{COOH}$) and the lower ester derivatives thereof ($-\text{COOR}$).

Detailed Description of the Preferred Embodiments

The peptides of this invention have an amino acid sequence selected from a group consisting of the 15 formulae I-X, supra, and the pharmaceutically acceptable salts thereof.

Preferably, the peptides of this invention have an amino acid sequence selected from the group consisting of the following formulae:

- | | | |
|----|---|-----------|
| 20 | X-Tyr-D-Trp-E ₁ -Trp-D-Phe-Q | (XI) |
| | X-Trp-D-Phe-Ala-Tyr-J ₂ -Q | (XII) |
| | X-Y ₃ -D-Phe-D-Phe-Lys-Met-Q | (XIII) |
| | X-D-Trp-Z ₄ -E ₄ -Q | (XIV) |
| | X-D-Lys-Tyr-D-Trp-D-Trp-Phe-Q | (XV) |
| 25 | X-Tyr-Gly-D-Trp-Phe-D-Phe-Q | (XVI) |
| | X-D-Phe-Z ₇ -E ₇ -Phe-J ₇ -Q | (XVII) |
| | X-Y ₈ -D-Trp-E ₈ -G ₈ -Q | (XVIII A) |
| | X-Tyr-D-Trp-D-Trp-Tyr-Q | (XVIII B) |
| | X-Y ₉ -D-Trp-E ₉ -G ₉ -J ₉ -Q | (XIX) |
| 30 | X'-Tyr-D-Trp-E ₁₀ -Trp-D-Phe-L ₁₀ -Q | (XX) |

wherein

X, X', Y₈, Y₉, and Q are as defined above;

E₄, Z₇, G₈, and G₉ are selected from the 35 group consisting of tryptophan and phenylalanine;

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Z_4 , E_7 , E_8 , and E_9 are selected from the group consisting of D-tryptophan and D-phenylalanine;

E_1 is selected from a group consisting of glycine, alanine, valine, leucine, isoleucine, 5 serine, threonine, asparagine, and glutamine;

J_2 is an amino acid residue selected from the group consisting of D-leucine and methionine;

Y_3 is an amino acid residue selected from a group consisting of tryptophan and D-tryptophan;

10 J_7 is selected from a group consisting of lysine and methionine;

J_9 is selected from the group consisting of methionine, D-methionine, leucine, D-leucine, phenylalanine, D-phenylalanine, arginine, D-arginine, 15 proline, and D-proline;

E_{10} is selected from a group consisting of glycine, alanine, valine, leucine, isoleucine, serine, threonine, asparagine, and glutamine; and

20 L_{10} is selected from the group consisting of arginine, lysine, asparagine, glutamine, serine, and threonine.

More preferably, the peptides of this invention have the amino acid sequence selected from the group consisting of the following formulae:

25 $X'-\text{Tyr-D-Trp-Ala-Trp-D-Phe-L}_{10}-Q$ (XXI)
wherein X' and Q are as defined above; and L_{10} is selected from the group glutamine and threonine.

All amino acid residues identified herein are in the natural or L-configuration unless otherwise 30 specified.

Abbreviations for amino acid residue have been used in accordance with the following standard peptide nomenclature:

Tyr	-L-tyrosine	Ile	-L-isoleucine
D-Tyr	-D-tyrosine	D-Ile	-D-isoleucine
Gly	-glycine	Leu	-L-leucine

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Phe	-L-phenylalanine	D-Leu	-D-leucine
D-Phe	-D-phenylalanine	Thr	-L-threonine
Met	-L-methionine	D-Thr	-D-threonine
D-Met	-D-methionine	Val	-L-valine
5 Ala	-L-alanine	D-Val	-D-valine
D-Ala	-D-alanine	Pro	-L-proline
Ser	-L-serine	D-Pro	-D-proline
D-Ser	-D-serine	Gln	-L-glutamine
Lys	-L-lysine	D-Gln	-D-glutamine
10 D-Lys	-D-lysine	Glu	-L-glutamic acid
Asn	-L-asparagine	D-Glu	-D-glutamic acid
D-Asn	-D-asparagine	Trp	-L-tryptophan
His	-L-histidine	D-Trp	-D-tryptophan
D-His	-D-histidine	D-Asp	-D-aspartic acid
15 Cys	-L-cysteine	Arg	-L-arginine
D-Cys	-D-cysteine	D-Arg	-D-arginine
Dopa	-L-dopamine	<Glu	-L-pyroglutamic acid
D-Dopa	-D-dopamine	D-<Glu	-D-pyroglutamic acid
Hypro	-L-hydroxyproline	D-Hypro	-D-hydroxyproline

20 The term "pharmaceutically acceptable salts," as used herein, refers to the non-toxic alkali metal, alkaline earth metal and ammonium salts commonly used in the pharmaceutical industry including the sodium, potassium, lithium, calcium, magnesium, barium, am-
25 monium and protamine zinc salts which are prepared by methods well known in the art. The term also includes non-toxic acid addition salts which are generally prepared by reacting the compounds of this invention with a suitable organic or inorganic acid. Representative
30 salts include the hydrochloride, hydrobromide, sulfate, bisulfate, acetate, oxalate, valerate, oleate, laurate, borate, benzoate, lactate, phosphate, tosylate, ci-
trate, maleate, fumarate, succinate, tartrate, nap-
sylate, and the like.

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The term "lower alkyl", as used herein, refers to straight and branched chain alkyl groups having from 1 to 6 carbon atoms, such as methyl, ethyl, n-propyl, iso-propyl, n-butyl, tert-butyl, sec-butyl, 5 n-pentyl, n-hexyl, 1,2-dimethylbutyl, and the like. Preferably, the lower alkyl group is methyl or ethyl.

The term "lower alkoxy", as used herein, refers to straight and branched chain alkoxy groups having from 1 to 6 carbon atoms, such as methoxy, 10 ethoxy, n-propoxy, iso-propoxy, n-butoxy, tert-butoxy, sec-butoxy, n-pentoxy, n-hexaoxy, 1,2-dimethylbutoxy and the like. Preferably, the lower alkoxy group is methoxy or ethoxy.

The term "lower ester derivative", as used 15 herein, refers to straight and branched chain alkyl ester derivatives having from 1 to 6 carbon atoms, such as methyl, ethyl, n-propyl, iso-propyl, n-butyl, tert-butyl, sec-butyl, n-pentyl, n-hexyl, and 1,2-dimethyl butyl ester derivatives and the like. Preferably, the 20 lower ester derivative is a methyl ester derivative or an ethyl ester derivative.

Accordingly, each R, R₁, and R₂ of Q is selected from a group consisting of straight and branched chain alkyl groups containing 1-6 carbon 25 atoms. Preferably, each R, R₁, and R₂ is selected from the group consisting of alkyl group containing 1-2 carbon atoms.

Peptides within the scope of the instant invention include, but are not limited to, those set 30 forth in Table I.

TABLE I

- X-Tyr-D-Trp-Ala-Trp-D-Phe-Q
- X-Tyr-D-Trp-Ser-Trp-D-Phe-Q
- X-Tyr-D-Trp-Asn-Trp-D-Phe-Q
- X-Tyr-D-Trp-Gln-Trp-D-Phe-Q
- X-Tyr-D-Trp-Thr-Trp-D-Phe-Q

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- X-Tyr-D-Trp-Gly-Trp-D-Phe-Q
X-Trp-D-Phe-Ala-Tyr-D-Leu-Q
X-Trp-D-Phe-Ala-Tyr-D-Leu-Q
X-Trp-D-Phe-Ala-Tyr-Met-Q
5 X-Trp-D-Phe-D-Phe-Lys-Met-Q
X-D-Trp-D-Phe-D-Phe-Lys-Met-Q
X-D-Trp-D-Trp-Trp-Q
X-D-Trp-D-Trp-Phe-Q
X-D-Trp-D-Phe-Trp-Q
10 X-D-Trp-D-Phe-Phe-Q
X-D-Lys-Tyr-D-Trp-D-Trp-Phe-CONH₂
X-D-Lys-Tyr-D-Trp-D-Trp-Phe-CONHR
X-D-Lys-Tyr-D-Trp-D-Trp-Phe-CONR₁R₂
X-D-Lys-Tyr-D-Trp-D-Trp-Phe-CH₂OR
15 X-D-Lys-Tyr-D-Trp-D-Trp-Phe-CH₂OH
X-D-Lys-Tyr-D-Trp-D-Trp-Phe-COON
X-D-Lys-Tyr-D-Trp-D-Trp-Phe-COOR
X-Tyr-Gly-D-Trp-Phe-D-Phe-CONH₂
X-Tyr-Gly-D-Trp-Phe-D-Phe-CONHR
20 X-Tyr-Gly-D-Trp-Phe-D-Phe-CONR₁R₂
X-Tyr-Gly-D-Trp-Phe-D-Phe-CH₂OR
X-Tyr-Gly-D-Trp-Phe-D-Phe-CH₂OH
X-Tyr-Gly-D-Trp-Phe-D-Phe-COOH
X-Tyr-Gly-D-Trp-Phe-D-Phe-COOR
25 X-D-Phe-Trp-D-Trp-Phe-Lys-Q
X-D-Phe-Trp-D-Trp-Phe-Met-Q
X-D-Phe-Trp-D-Phe-Phe-Lys-Q
X-D-Phe-Trp-D-Phe-Phe-Met-Q
X-D-Phe-Phe-D-Trp-Phe-Lys-Q
30 X-D-Phe-Phe-D-Trp-Phe-Met-Q
X-D-Phe-Phe-D-Phe-Phe-Lys-Q
X-D-Phe-Phe-D-Phe-Phe-Met-Q
X-Tyr-D-Trp-D-Trp-Trp-Q
X-Tyr-D-Trp-D-Trp-Phe-Q
35 X-Tyr-D-Trp-D-Phe-Trp-Q
X-Trp-D-Trp-D-Trp-Trp-Q

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		X-Trp-D-Trp-D-Trp-Phe-Q
		X-Trp-D-Trp-D-PheTrp-Q
		X-Trp-D-Trp-D-Phe-Phe-Q
		X-Phe-D-Trp-D-Trp-Trp-Q
5		X-Phe-D-Trp-D-Trp-Phe-Q
		X-Phe-D-Trp-D-Phe-Trp-Q
		X-Phe-D-Trp-D-Phe-Phe-Q
		X-Tyr-D-Trp-D-Trp-Tyr-CONH ₂
		X-Tyr-D-Trp-D-Trp-Tyr-CONHR
10		X-Tyr-D-Trp-D-Trp-Tyr-CONR ₁ R ₂
		X-Tyr-D-Trp-D-Trp-Tyr-CH ₂ OR
		X-Tyr-D-Trp-D-Trp-Tyr-CH ₂ OH
		X-Tyr-D-Trp-D-Trp-Tyr-COOH
		X-Tyr-D-Trp-D-Trp-Tyr-COOR
		X-Tyr-D-Trp-D-Trp-Trp-Met-Q
15		X-Tyr-D-Trp-D-Trp-Trp-D-Met-Q
		X-Tyr-D-Trp-D-Trp-Trp-Leu-Q
		X-Tyr-D-Trp-D-Trp-Trp-D-Leu-Q
		X-Tyr-D-Trp-D-Trp-Trp-Phe-Q
		X-Tyr-D-Trp-D-Trp-Trp-D-Phe-Q
20		X-Tyr-D-Trp-D-Trp-Trp-Arg-Q
		X-Tyr-D-Trp-D-Trp-Trp-D-Arg-Q
		X-Tyr-D-Trp-D-Trp-Trp-Pro-Q
		X-Tyr-D-Trp-D-Trp-Trp-D-Pro-Q
		X-Tyr-D-Trp-D-Trp-Phe-Met-Q
25		X-Tyr-D-Tr-D-Trp-Phe-D-Met-Q
		X-Tyr-D-Trp-D-Trp-Phe-Leu-Q
		X-Tyr-D-Trp-D-Trp-Phe-D-Leu-Q
		X-Tyr-D-Trp-D-Trp-Phe-Phe-Q
		X-Tyr-D-Trp-D-Trp-Phe-D-Phe-Q
30		X-Tyr-D-Trp-D-Trp-Phe-Arg-Q
		X-Tyr-D-Trp-D-Trp-Phe-D-Arg-Q
		X-Tyr-D-Trp-D-Trp-Phe-Pro-Q
		X-Tyr-D-Trp-D-Trp-Phe-D-Pro-Q
		X-Tyr-D-Trp-D-Phe-Trp-Met-Q
35		X-Tyr-D-Trp-D-Phe-Trp-D-Met-Q

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X-Tyr-D-Trp-D-Phe-Trp-Leu-Q
X-Tyr-D-Trp-D-Phe-Trp-D-Leu-Q
X-Tyr-D-Trp-D-Phe-Trp-Phe-Q
X-Tyr-D-Trp-D-Phe-Trp-D-Phe-Q
5 X-Tyr-D-Trp-D-Phe-Trp-Arg-Q
X-Tyr-D-Trp-D-Phe-Trp-D-Arg-Q
X-Tyr-D-Trp-D-Phe-Trp-Pro-Q
X-Tyr-D-Trp-D-Phe-Trp-D-Pro-Q
X-Tyr-D-Trp-D-Phe-Phe-Met-Q
10 X-Tyr-D-Trp-D-Phe-Phe-D-Met-Q
X-Tyr-D-Trp-D-Phe-Phe-Leu-Q
X-Tyr-D-Trp-D-Phe-Phe-D-Leu-Q
X-Tyr-D-Trp-D-Phe-Phe-Phe-Q
X-Tyr-D-Trp-D-Phe-Phe-D-Phe-Q
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X-Tyr-D-Trp-D-Phe-Phe-Pro-Q
X-Tyr-D-Trp-D-Phe-Phe-D-Pro-Q
X-Trp-D-Trp-D-Trp-Trp-Met-Q
20 X-Trp-D-Trp-D-Trp-Trp-D-Met-Q
X-Trp-D-Trp-D-Trp-Trp-Leu-Q
X-Trp-D-Trp-D-Trp-Trp-D-Leu-Q
X-Trp-D-Trp-D-Trp-Trp-Phe-Q
X-Trp-D-Trp-D-Trp-Trp-D-Phe-Q
25 X-Trp-D-Trp-D-Trp-Trp-Arg-Q
X-Trp-D-Trp-D-Trp-Trp-D-Arg-Q
X-Trp-D-Trp-D-Trp-Trp-Pro-Q
X-Trp-D-Trp-D-Trp-Trp-D-Pro-Q
X-Trp-D-Trp-D-Trp-Phe-Met-Q
30 X-Trp-D-Trp-D-Trp-Phe-D-Met-Q
X-Trp-D-Trp-D-Trp-Phe-Leu-Q
X-Trp-D-Trp-D-Trp-Phe-D-Leu-Q
X-Trp-D-Trp-D-Trp-Phe-Phe-Q
X-Trp-D-Trp-D-Trp-Phe-D-Phe-Q
35 X-Trp-D-Trp-D-Trp-Phe-Arg-Q
X-Trp-D-Trp-D-Trp-Phe-D-Arg-Q

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X-Trp-D-Trp-D-Trp-Phe-Pro-Q
 X-Trp-D-Trp-D-Trp-Phe-D-Pro-Q
 X-Trp-D-Trp-D-Phe-Trp-Met-Q
 X-Trp-D-Trp-D-Phe-Trp-D-Met-Q
 X-Trp-D-Trp-D-Phe-Trp-Leu-Q
 5 X-Trp-D-Trp-D-Phe-Trp-D-Leu-Q
 X-Trp-D-Trp-D-Phe-Trp-Phe-Q
 X-Trp-D-Trp-D-Phe-Trp-D-Phe-Q
 X-Trp-D-Trp-D-Phe-Trp-Arg-Q
 X-Trp-D-Trp-D-Phe-Trp-D-Arg-Q
 10 X-Trp-D-Trp-D-Phe-Trp-Pro-Q
 X-Trp-D-Trp-D-Phe-TrpD-Pro-Q
 X-Trp-D-Trp-D-Phe-Phe-Met-Q
 X-Trp-D-Trp-D-Phe-Phe-D-Met-Q
 X-Trp-D-Trp-D-Phe-Phe-Leu-Q
 15 X-Trp-D-Trp-D-Phe-Phe-D-Leu-Q
 X-Trp-D-Trp-D-Phe-Phe-Arg-Q
 X-Trp-D-Trp-D-Phe-Phe-D-Arg-Q
 X-Trp-D-Trp-D-Phe-Phe-Pro-Q
 X-Trp-D-Trp-D-Phe-Phe-D-Pro-Q
 20 X-Phe-D-Trp-D-Trp-Trp-Met-Q
 X-Phe-D-Trp-D-Trp-Trp-D-Met-Q
 X-Phe-D-Trp-D-Trp-Trp-Leu-Q
 X-Phe-D-Trp-D-Trp-Trp-D-Leu-Q
 X-Phe-D-Trp-D-Trp-Trp-Phe-Q
 X-Phe-D-Trp-D-Trp-Trp-D-Phe-Q
 X-Phe-D-Trp-D-Trp-Trp-Arg-Q
 25 X-Phe-D-Trp-D-Trp-Trp-D-Arg-Q
 X-Phe-D-Trp-D-Trp-Trp-Pro-Q
 X-Phe-D-Trp-D-Trp-Trp-D-Pro-Q
 X-Phe-D-Trp-D-Trp-Trp-Phe-Met-Q
 X-Phe-D-Trp-D-Trp-Phe-D-Met-Q
 X-Phe-D-Trp-D-Trp-Phe-Leu-Q
 X-Phe-D-Trp-D-Trp-Phe-D-Leu-Q
 X-Phe-D-Trp-D-Trp-Phe-Phe-Q
 30 X-Phe-D-Trp-D-Trp-Phe-D-Phe-Q
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X-Phe-D-Trp-D-Trp-Phe-Arg-Q
X-Phe-D-Trp-D-Trp-Phe-D-Arg-Q
X-Phe-D-Trp-D-Trp-Phe-Pro-Q
X-Phe-D-Trp-D-Trp-Phe-D-Pro-Q
5 X-Phe-D-Trp-D-Phe-TrpMet-Q
X-Phe-D-Trp-D-Phe-Trp-D-Met-Q
X-Phe-D-Trp-D-Phe-Trp-Leu-Q
X-Phe-D-Trp-D-Phe-Trp-Phe-Q
X-Phe-D-Trp-D-Phe-Trp-D-Phe-Q
10 X-Phe-D-Trp-D-Phe-Trp-Arg-Q
X-Phe-D-Trp-D-Phe-Trp-D-Arg-Q
X-Phe-D-Trp-D-Phe-Trp-Pro-Q
X-Phe-D-Trp-D-Phe-Trp-D-Pro-Q
X-Phe-D-Trp-D-Phe-Phe-Met-Q
15 X-Phe-D-Trp-D-Phe-Phe-D-Met-Q
X-Phe-D-Trp-D-Phe-Phe-Leu-Q
X-Phe-D-Trp-D-Phe-Phe-D-Leu-Q
X-Phe-D-Trp-D-Phe-Phe-Phe-Q
X-Phe-D-Trp-D-Phe-Phe-D-Phe-Q
20 X-Phe-D-Trp-D-Phe-Phe-Arg-Q
X-Phe-D-Trp-D-Phe-Phe-D-Arg-Q
X-Phe-D-Trp-D-Phe-Phe-Pro-Q
X-Phe-D-Trp-D-Phe-Phe-D-Pro-Q
X'-Tyr-D-Trp-Ala-Trp-D-Phe-Gln-Q
25 X'-Tyr-D-Trp-Gly-Trp-D-Phe-Gln-Q
X'-Tyr-D-Trp-Ala-Trp-D-Phe-Thr-Q
X'-Phe-D-Phe-Ser-Phe-D-Phe-Asn-Q
X'-Trp-D-Tyr-Met-Tyr-D-Trp-Ser-Q
X'-Tyr-D-Trp-Asp-Trp-D-Tyr-Lys-Q
30 X'-Trp-D-Trp-Val-Trp-D-Trp-Glu-Q
X'-Tyr-D-Tyr-Leu-Trp-D-Trp-Arg-Q
X'-Phe-D-Trp-Ile-Trp-D-Phe-Gln-Q
X'-Tyr-D-Phe-Ala-Phe-D-Phe-Arg-Q
X'-Tyr-D-Trp-Gly-Trp-D-Tyr-Thr-Q
35 X'-Tyr-D-Tyr-Val-Tyr-D-Phe-Glu-Q
X'-Trp-D-Trp-Leu-Trp-D-Phe-Asn-Q

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X'-Tyr-D-Trp-Ile-Trp-D-Trp-Lys-Q

X'-Tyr-D-Trp-Ala-Trp-D-Phe-Asn-Q

X'-Tyr-D-Trp-Ala-Trp-D-Phe-Ser-Q

X'-Tyr-D-Trp-Ala-Trp-D-Phe-Lys-Q

5

X'-Tyr-D-Trp-Ala-Trp-D-Phe-Glu-Q

The peptides of the instant invention can be prepared by classical methods known in the art or, preferably, by using standard solid-phase techniques.

The synthesis, for example, can be commenced from the 10 C-terminal end of the peptide using an α -amino protected amino acid. A suitable starting material can be prepared, for instance, by attaching the required α -amino acid to a chloromethylated resin, a hydroxymethyl resin, or a benzhydrylamine resin. One such 15 chloromethylated resin is sold under the tradename BIO-BEADS SX-1 by Bio Rad Laboratories, Richmond, California and the preparation of the hydroxymethyl resin is described by Bodanszky et al., Chem. Ind. (London) 38, 1597 (1966). The benzhydrylamine (BHA) 20 resin has been described by Pietta and Marshall, Chem. Commn. 650 (1970) and is commercially available from Beckman Instruments, Inc., Palo Alto, California in the hydrochloride form thereof (BHA-HCl).

In the preparation of the compounds of this 25 invention, an α -amino protected amino acid can be coupled to the chloromethylated resin with the aid of, for example, cesium bicarbonate catalyst, according to the method described by Gisin, Helv. Chim. Acta, 56, 1467 (1973). After the initial coupling, the α -amino 30 protecting group can be removed by a choice of reagents including trifluoroacetic acid (TFA) or hydrochloric acid (HCl) solutions in organic solvents at room temperature. After removal of the α -amino protecting group, the remaining protected amino acids can be 35 coupled stepwise in the desired order. Each protected amino acid can be generally reacted in about a 3-fold

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excess using an appropriate carboxyl group activator such as dicyclohexylcarbodiimide (DCC) in solution, for example, in methylene chloride(CH_2Cl_2)-dimethylformamide (DMF) mixtures.

5 After the desired amino acid sequence has been completed, the desired peptide can be decoupled from the resin support by treatment with a reagent such as hydrogen fluoride (HF) which not only cleaves the peptide from the resin, but also cleaves all remaining 10 side-chain protecting groups. When the chloromethylated resin is used, hydrogen fluoride treatment results in the formation of the free peptide acids of Formula I ($\text{Y} = -\text{COOH}$). When the benzhydrylamine resin is used, hydrogen fluoride treatment results directly in the 15 free peptide amides of Formula I ($\text{Y} = -\text{CONH}_2$). Alternatively, when the chloromethylated or hydroxy-methylated resin is employed, the side-chain protected peptide can be decoupled by treatment of the peptide-resin with ammonia to give the desired side-chain 20 protected amide or with an alkylamine to give a side-chain protected alkylamide or dialkylamide. Side-chain protection can then be removed in the usual fashion by treatment with hydrogen fluoride to give the free amides, alkylamides, or dialkylamides.

25 In preparing the esters of this invention, the resins used to prepare the acids of Formula I ($\text{Y} = -\text{COOH}$) can be employed and the side-chain protected peptide can be cleaved with base and the appropriate alcohol, i.e., methanol. Side-chain protecting groups 30 can then be removed in the usual fashion by treatment with hydrogen fluoride to obtain the desired ester.

The solid-phase procedure discussed above is well known in the art and has been essentially described by J. M. Stewart, Solid Phase Peptide Synthesis: (Freeman and Co., San Francisco, 1969).

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The growth hormone releasing pentapeptides of Formula I are useful in vitro as unique tools for understanding how growth hormone secretion is regulated at the pituitary level. This includes use in the 5 evaluation of many factors thought or known to influence growth hormone secretion such as age, sex, nutritional factors, glucose, amino acids, fatty acids, as well as fasting and non-fasting states. In addition, the peptides of this invention can be used in the 10 evaluation of how other hormones modify growth hormone releasing activity. For example, it has already been established that somatostatin inhibits growth hormone release. Other hormones that are important and in need of study as to their effect on growth hormone release 15 include the gonadal hormones testosterone, estradiol, and progesterone; the adrenal hormones cortisol and other corticoids, epinephrin and norepinephrine; the pancreatic and gastrointestine hormones, insulin, glucagon, gastric, secretion, the vasoactive intestinal 20 peptides, i.e., bombesin; and the thyroid hormones thyroxine and triiodothyronine. The peptides of the instant invention can also be employed to investigate the possible negative or positive feedback effects of some of the pituitary hormones, e.g., growth hormone 25 and endorphin peptides, on the pituitary to modify growth hormone release. Of particular scientific importance is the use of these peptides to elucidate the subcellular mechanisms mediating the release of growth hormone.

30 The peptides of the present invention can also be administered to warm blooded animals, including man, to release growth hormone in vivo. For example, the peptides can be administered to treat symptoms related to growth hormone deficiencies. In addition, 35 these peptides can be administered to commercially important animals to accelerate and increase their rate and extent of growth.

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Accordingly, the present invention includes within its scope pharmaceutical compositions comprising, as an active ingredient, at least one of the peptides within the scope of this invention in association with a pharmaceutical carrier or diluent. The compounds of this invention can be administered by oral, parenteral (intramuscular, intraperitoneal, intravenous (IV) or subcutaneous injection), nasal, vaginal, rectal or sublingual routes of administration and can be formulated in dosage forms appropriate for each route of administration.

Solid dosage forms for oral administration include capsules, tablets, pills, powders and granules. In such solid dosage forms, the active compound is admixed with at least one inert pharmaceutically acceptable carrier such as sucrose, lactose, or starch. Such dosage forms can also comprise, as is normal practice, additional substances other than inert diluents, e.g., lubricating agents such as magnesium stearate. In the case of capsules, tablets and pills, the dosage forms may also comprise buffering agents. Tablets and pills can additionally be prepared with enteric coatings.

Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, solutions, suspensions, syrups, the elixirs containing inert diluents commonly used in the art, such as water. Besides, such inert diluents, compositions can also include adjuvants, such as wetting agents, emulsifying and suspending agents, and sweetening, flavoring, and perfuming agents.

Preparations according to this invention for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, or emulsions. Examples of non-aqueous solvents or vehicles are propylene glycol, polyethylene glycol, vegetable oils,

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such as olive oil and corn oil, gelatin, and injectable organic esters such as ethyl oleate. Such dosage forms may also contain adjuvants such as preserving, wetting, emulsifying, and dispersing agents. They may be sterilized by, for example, filtration through a bacteria-retaining filter, by incorporating sterilizing agents into the compositions, by irradiating the compositions, or by heating the compositions. They can also be manufactured in the form of sterile solid compositions which can be dissolved in sterile water, or some other sterile injectable medium immediately before use.

Compositions for rectal or vaginal administration are preferably suppositories which may contain, in addition to the active substance, excipients such as cocoa butter or a suppository wax.

Compositions for nasal or sublingual administration are also prepared with standard excipients well known in the art.

The dosage of active ingredient in the compositions of this invention may be varied; however, it is necessary that the amount of the active ingredient shall be such that a suitable dosage form is obtained. The selected dosage depends upon the desired therapeutic effect, on the route of administration, and on the duration of the treatment. Generally, dosage levels of between 0.001 to 10 mg/kg. of body weight daily are administered to mammals to obtain effective release of growth hormone.

The following examples are provided for the purpose of further illustration only and are not intended to be limitations on the disclosed invention.

Example 1

Synthesis of H₂N-Tyr-D-Trp-Ala-Trp-D-Phe-Gln-CONH₂

BHA-HCl resin was placed in a reaction vessel. The following procedure was then employed in conjunction with a Beckman brand Peptide Synthesizer

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Model No. 990 in preparing the hexapeptide $H_2N\text{-Tyr-D-Trp-Ala-Trp-D-Phe-Gln-CONH}_2$:

1. Methylene chloride (CH_2Cl_2 ; about 10 ml/gm of BHA·HCl resin) was added to the reaction vessel. The 5 BHA·HCl resin was washed with vigorous stirring for about 1.5 minutes. The CH_2Cl_2 solution was then drained from the reaction vessel. This washing step was repeated once.

2. A triethylamine solution ($(Et_3N)/CH_2Cl_2$ 10 (10:90); about 10 ml/gm BHA·HCl resin) was added to the washed BHA·HCl resin in the reaction vessel. The resulting mixture was vigorously stirred for about 1.5 minutes. The solution was then drained from the reaction vessel.

15 3. Another Et_3N_3/CH_2Cl_2 (10:90) solution (about 10 ml/gm BHA·HCl) was added to the reaction vessel. The BHA·HCl resin was neutralized by vigorous stirring for about 20 minutes. The solution was then drained from the reaction vessel.

20 4. CH_2Cl_2 (about 10 ml/gm of BHA·HCl resin) was added to the reaction vessel. The resulting mixture was vigorously stirred for about 1.5 minutes. The solution was then drained from the reaction vessel. This procedure was repeated an additional two times.

25 5. Tertiarybutyloxycarbonyl-glutamine (Boc-Gln; about 2.5 times the theoretical amount of the total binding capacity of the BHA·HCl resin originally placed in the reaction vessel) in about 50 ml of dimethyl-formamide-methylene chloride solution ($DMF-CH_2Cl_2$ 30 (1:9)) was added to the reaction vessel. The resulting mixture was vigorously stirred for about 1.5 minutes.

35 6. A 0.5 molar (M) dicyclohexylcarbodiimide (DCC) in CH_2Cl_2 solution (about 2.5 times the theoretical amount of total binding capacity of the BHA·HCl resin originally placed in the reaction vessel) was added to the reaction vessel. The resulting mixture

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was vigorously stirred until a negative ninhydrin test was obtained (about 120 minutes). The solution was then drained from the reaction vessel.

7. CH_2Cl_2 (about 10 ml/gm of BHA·HCl resin) was
5 added to the reaction vessel. The resulting solution was vigorously stirred for about 1.5 minutes. The solution was then drained from the reaction vessel. This washing procedure was repeated once.

8. DMF (about 10 ml/gm of BHA·HCl resin) was
10 added to the reaction vessel. The resulting mixture was stirred for about 1.5 minutes. The solution was then drained from the reaction vessel.

9. CH_2Cl_2 (about 10 ml/gm of BHA·HCl resin) was added to the reaction vessel. The resulting mixture
15 was vigorously stirred for about 1.5 minutes. The solution was then drained from the reaction vessel. This washing procedure was repeated an additional two times.

10. A trifluoroacetic acid/methylene chloride
20 solution (TFA/ CH_2Cl_2 (40:60); about 10 ml/gm of BHA·HCl resin) was added to the reaction vessel. The resulting mixture was vigorously stirred for about 1.5 minutes. The solution was then drained from the reaction vessel.

11. Another TFA/ CH_2Cl_2 (40:60) solution (about 10
25 ml/gm of BHA·HCl resin) was added to the reaction vessel. The resulting mixture was vigorously stirred for about 20 minutes. The solution was then drained from the reaction vessel.

12. CH_2Cl_2 (about 10 ml/gm of BHA·HCl resin) was
30 added to the reaction vessel. The resultig solution was vigorously stirred for about 1.5 minutes. The solution was then drained from the reaction vessel. This washing procedure was repeated once.

13. A triethylamine solution ($(\text{Et}_3\text{N})/\text{CH}_2\text{Cl}_2$
35 (10:90); about 10 ml/gm BHA·HCl resin) was added to the washed BHA·HCl resin in the reaction vessel. The

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resulting mixture was vigorously stirred for about 1.5 minutes. The solution was then drained from the reaction vessel.

14. Another $\text{Et}_2\text{N}_3/\text{CH}_2\text{Cl}_2$ (10:90) solution (about 5 10 ml/gm BHA·HCl) was added to the reaction vessel. The BHA·HCl resin was neutralized by vigorous stirring for about 20 minutes. The solution was then drained from the reaction vessel.

15. Chloroform (CHCl_3 ; about 10 ml/gm of BHA·HCl 10 resin) was added to the reaction vessel. The resulting mixture was vigorously stirred for about 1.5 minutes. The solution was then drained from the reaction vessel.

16. An ethanol/methylene chloride solution (EtoH/ CH_2Cl_2 (30:70); about 10 ml/gm of BHA·HCl resin) 15 was added to the reaction vessel. The resulting mixture was vigorously stirred for about 1.5 minutes. The solution was then drained from the reaction vessel. This washing step was repeated once.

Steps 4 through 16 were then repeated employing the following sequence of amino acids:

Boc-D-Phe

Boc-Trp

Boc-Ala

Boc-D-Trp

25 Boc-Tyr (BrZ*)

*BrZ denotes p-bromobenzylloxycarbonyl

After completion of the synthesis of the desired peptide resin, the reaction vessel containing the peptide resin was then placed in a dessicator and 30 dried overnight under a vacuum. The dried peptide resin was removed from the reaction vessel and placed in another vessel suitable for HF cleavage. This latter vessel also contained a magnetic stirring bar. A quantity of anisole sufficient to wet the peptide 35 resin was added to this vessel. The vessel was next connected to an HF line and placed under a vacuum to

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remove any air therein. The vessel was then cooled to about -78°C. with a dry ice-acetone bath. Doubly distilled HF (about 10 ml/gm of peptide resin) was added to the vessel. The dry ice-acetone bath was then removed from the vessel and replaced by an ice-water bath. The vessel's contents were vigorously stirred for about 45 minutes while the vessel remained immersed in the ice-water bath. Most of the HF in the vessel was then removed by water aspiration. After the majority of HF was removed by water aspiration, the remaining HF and anisole were removed via a vacuum pump.

The vessel's contents were washed with about 100 ml of ether to further remove any residual anisole.

The peptide was removed from the resin by extraction with 30% aqueous acetic acid (aq.HOAc). The aq.HOAc was lyophilized off to yield a fluffy peptide powder.

The peptide was then purified by partition chromatography or counter current distribution (CCD) employing a butanol: HOAc: water (4:1:5) system. When further purification was necessary, a Pharmacia LH-20 brand chromatography column was also employed.

Example 2

Synthesis of H₂N-Tyr-D-Trp-Ala-Trp-D-Phe-Thr-CONH₂

25 The procedure set forth in Example 1 was employed to synthesize the hexapeptide H₂N-Tyr-D-Trp-Ala-Trp-D-Phe-Thr-CONH₂ employing the following sequence of amino acids:

30	Boc-Thr
	Boc-D-Phe
	Boc-Trp
	Boc-Ala
	Boc-D-Trp
	Boc-Tyr(Bz)

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Example 3

Synthesis of H₂N-Tyr-D-Trp-Ala-Trp-D-Phe-Gln-COOH

The procedure set forth in Example 1 was employed with several modifications to synthesize the 5 hexapeptide H₂N-Tyr-D-Trp-Ala-Trp-D-Phe-Gln-COOH employing the following sequence of amino acids:

	Boc-Gln
	Boc-D-Phe
	Boc-Trp
10	Boc-Ala
	Boc-D-Trp
	Boc-Tyr(BrZ)

The modifications consisted of:

(1) Employing a hydroxymethyl resin in place of 15 BHA·HCl resin.

(2) Omitting steps 2-4 of Example 1 and replacing them with a single step which entailed adding N,N-dimethylaminopyridine (about 2.5 times the theoretical amount of total binding capacity of the hydroxymethyl 20 resin originally placed in the reaction vessel) to the reaction vessel. The resulting mixture was vigorously stirred for about 1.5 minutes. The solution was then drained from the reaction vessel.

(3) All calculations, where applicable, were 25 based on the amount of hydroxymethyl resin (instead of BHA·HCl resin) originally placed in the reaction vessel.

Example 4

Synthesis of H₂N-Tyr-D-Trp-Ala-Trp-D-Phe-CONH₂

30 The procedure set forth in Example 1 was employed with several modifications to synthesize the pentapeptide H₂N-Tyr-D-Trp-Ala-Trp-D-Phe-CONH₂ employing the following sequence of amino acids:

	Boc-D-Phe
35	Boc-Trp

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Boc-Ala
 Boc-D-Trp
 Boc-Tyr-BrZ)

The modifications consisted of:

5 (1) Substituting the following steps for steps 5 and 6 of Example 1:

5. An 0.5 molar (M) dicyclohexylcarbodiimide (DCC) in CH_2Cl_2 solution (about 2.5 times the theoretical amount of total binding capacity of the BHA-HCl resin originally placed in the reaction vessel) was added to the reaction vessel. The resulting mixture was vigorously stirred for about 1.5 minutes. The solution was then drained from the reaction vessel.

6. Tertiarybutyloxycarbonyl-D-phenylalanine
 15 (Boc-D-Phe; about 2.5 times the theoretical amount of the total binding capacity of the BHA-HCl resin originally placed in the reaction vessel) in about 50 ml of DMF- CH_2Cl_2 (5:45) solution was added to the reactin vessel. The resulting mixture was vigorously stirred
 20 until a negative ninhydrin test was obtained (about 120 minutes). The solution was then drained from the reaction vessel.

(2) Omitting steps 12-14 of Example 1.

(3) After employing steps 1-16 of Example 1, as
 25 modified herein by (1) and (2) of this Example 4, with respect to the first amino acid, Boc-D-Phe, these modified steps 1-16 were then repeated for the remaining amino acids of the above sequence.

4. The peptide was purified by partition chromatography employing a butanol: HOAc: water (4:1:5)
 30 system.

Example 5

Synthesis of $\text{H}_2\text{N-Tyr-D-Trp-Asn-Trp-D-Phe-CONH}_2$

The procedure set forth in Example 4 was
 35 employed with one modification to synthesize the penta-

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peptide $\text{H}_2\text{N-Tyr-D-Trp-Asn-Trp-D-Phe-CONH}_2$ employing the following sequence of amino acids:

Boc-D-Phe
 Boc-Trp
 5 Boc-Asn-ONP*
 Boc-D-Trp
 Boc-Tyr(BrZ)

*ONP denotes para-nitrophenylester.

The sole modification entailed in the omission of step 10.5 prior to the addition of Boc-Asn-ONP to the intermediate peptide resin.

Example 6

Synthesis of $\text{H}_2\text{N-Tyr-D-Trp-Ala-Trp-D-Phe-COOH}$

The procedure set forth in Example 4 was 15 employed with several modifications to synthesize the pentapeptide $\text{H}_2\text{N-Tyr-D-Trp-Ala-Trp-D-Phe-COOH}$ employing the following sequence of amino acids:

Boc-D-Phe
 Boc-Trp
 20 Boc-Ala
 Boc-D-Trp
 Boc-Tyr(BrZ)

The modifications consisted of:

(1) Employing a hydroxymethyl resin in place of 25 BHA-HCl resin.

(2) Omitting steps 2-4 of Example 4 and replacing them with a single step which entailed adding N,N-dimethylaminopyridine (about 2.5 times the theoretical amount of total binding capacity of the hydroxymethyl resin originally placed in the reaction vessel) to the reaction vessel. The resulting mixture was vigorously stirred for about 1.5 minutes. The solution was then drained from the reaction vessel.

(3) All calculations, where applicable, were 35 based on the amount of hydroxymethyl resin (instead of

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BHA-HCl resin) originally placed in the reaction vessel.

Example 7

Synthesis of H₂N-Tyr-D-Trp-Ser-Trp-D-Phe-CONH₂

5 The procedure set forth in Example 4 was employed to synthesize the pentapeptide H₂N-Tyr-D-Trp-Ser-Trp-D-Phe-CONH₂ employing the following sequence of amino acids:

Boc-D-Phe
10 Boc-Trp
 Boc-Ser(Bz)*
 Boc-D-Trp
 Boc-Tyr(BrZ)

*Bz denotes benzyl

Example 8

Synthesis of H₂N-Trp-D-Phe-Ala-Tyr-D-Leu-CONH₂

15 The procedure set forth in Example 4 was employed to synthesize the pentapeptide H₂N-Trp-D-Phe-Ala-Tyr-D-Leu-CONH₂ employing the following sequence of 20 amino acids:

Boc-D-Leu
25 Boc-Tyr(BrZ)
 Boc-Ala
 Boc-D-Phe
 Boc-Trp

Example 9

Synthesis of H₂N-Trp-D-Phe-Ala-Tyr-Met-CONH₂

30 The procedure set forth in Example 4 was employed to synthesize the pentapeptide H₂N-Trp-D-Phe-Ala-Tyr-Met-CONH₂ employing the following sequence of amino acids:

Boc-Met
 Boc-Tyr(BrZ)
 Boc-Ala

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Boc-D-Phe

Boc-Trp

Example 10Synthesis of H₂N-D-Trp-D-Phe-D-Phe-Lys-Met-CONH₂

5 The procedure set forth in Example 4 was employed to synthesize the pentapeptide H₂N-D-Trp-D-Phe-D-Phe-Lys-Met-CONH₂ employing the following sequence of amino acids:

10 Boc-Met
 Boc-Lys(Z)*
 Boc-D-Phe
 Boc-D-Phe
 Boc-D-Trp

*Z denotes benzyloxycarbonyl; see Example 12, infra,
 15 for preparation of Boc-Lys(Z).

Example 11Synthesis of H₂N-Trp-D-Phe-D-Phe-Lys-Met-CONH₂

The procedure set forth in Example 4 was employed to synthesize the pentapeptide H₂N-Trp-D-Phe-
 20 D-Phe-Lys-Met-CONH₂ employing the following sequence of amino acids:

25 Boc-Met
 Boc-Lys(Z)
 Boc-D-Phe
 Boc-D-Phe
 Boc-Trp

Example 12Preparation of Boc-Lys(Z)

Into a separatory funnel was placed the di-
 30 cyclohexyl ammonium salt (DCHA) of Boc-Lys(Z)(Boc-Lys(Z)·DCHA; about 3 times the theoretical amount of the total binding capacity of the BHA·HCl resin originally placed in the reaction vessel in Example 1). About 40 ml of ethyl acetate (EtOAc) was next added to
 35 the separatory funnel. Sulfuric acid (H₂SO₄; 1N) in an

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amount sufficient to acidify the solid Boc-Lys(Z)-DCHA was then added to the separatory funnel. The separatory funnel and its contents were shaken vigorously and afterwards the EtOAc and H₂O layers were 5 allowed to separate. The H₂O layer was drained from the separatory funnel. H₂O was added to the separatory funnel and the shaking, standing, draining cycle was repeated. A sufficient amount of sodium sulfate (Na₂SO₄) to absorb any residual H₂O remaining in the 10 EtOAc was added to the EtOAc solution of Boc-Lys(Z). The resulting mixture was then filtered to remove the Na₂SO₄ therefrom. The filtered solution was then employed in the synthesis procedure set forth in Example 10, supra.

15 An analogous procedure was also employed to prepare the Boc-Lys(Z) used in Example 11, supra, and Examples 14 and 16, infra.

Example 13

Synthesis of H₂N-D-Trp-D-Trp-Phe-CONH₂

20 The procedure set forth in Example 4 was employed to synthesize the tripeptide H₂N-D-Trp-D-Trp-Phe-CONH₂ employing the following sequence of amino acids:

25 Boc-Phe
 Boc-D-Trp
 Boc-D-Trp

Example 14

Synthesis of H₂N-D-Lys-Tyr-D-Trp-D-Trp-Phe-CONH₂

The procedure set forth in Example 4 was 30 employed to synthesize the pentapeptide H₂N-D-Lys-Tyr-D-Trp-D-Trp-Phe-CONH₂ employing the following sequence of amino acids:

Boc-Phe
Boc-D-Trp

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Boc-D-Trp
 Boc-Tyr(BrZ)
 Boc-D-Lys(Z)

Example 15

5 Synthesis of H₂N-Tyr-Gly-D-Trp-Phe-D-Phe-CONH₂

The procedure set forth in Example 4 was employed to synthesize the pentapeptide H₂N-Tyr-Gly-D-Trp-Phe-D-Phe-CONH₂ employing the following sequence of amino acids:

10 Boc-D-Phe
 Boc-Phe
 Boc-D-Trp
 Boc-Gly
 Boc-Tyr(BrZ)

15 Example 16

Synthesis of H₂N-D-Phe-Trp-D-Phe-Phe-Lys-CONH₂

The procedure set forth in Example 4 was employed to synthesize the pentapeptide H₂N-D-Phe-Trp-D-Phe-Phe-Lys-CONH₂ employing the following sequence of 20 amino acids:

20 Boc-Lys(Z)
 Boc-Phe
 Boc-D-Phe
 Boc-Trp
 Boc-D-Phe

25 Example 17

Synthesis of H₂N-D-Phe-Trp-D-Phe-Phe-Met-CONH₂

The procedure set forth in Example 4 was employed to synthesize the pentapeptide H₂N-D-Phe-Trp-D-Phe-Phe-Met-CONH₂ employing the following sequence of 30 amino acids:

20 Boc-Met
 Boc-Phe
 Boc-D-Phe

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Boc-Trp

Boc-D-Phe

Example 18

Synthesis of H₂N-D-Phe-Phe-D-Trp-Phe-Met-CONH₂

5 The procedure set forth in Example 4 was employed to synthesize the pentapeptide H₂N-D-Phe-Phe-D-Trp-Phe-Met-CONH₂ employing the following sequence of amino acids:

Boc-Met

10 Boc-Phe
 Boc-D-Trp
 Boc-Phe
 Boc-D-Phe

Example 19

15 Synthesis of H₂N-Tyr-D-Trp-D-Trp-Phe-CONH₂

The procedure set forth in Example 4 was employed to synthesize the tetrapeptide H₂N-Tyr-D-Trp-D-Trp-Phe-CONH₂ employing the following sequence of amino acids:

20 Boc-Phe
 Boc-D-Trp
 Boc-D-Trp
 Boc-Tyr(BrZ)

Example 20

25 Synthesis of H₂N-Tyr-D-Trp-D-Trp-Tyr-CONH₂

The procedure set forth in Example 4 was employed to synthesize the tetrapeptide H₂N-Tyr-D-Trp-D-Trp-Tyr-CONH₂ employing the following sequence of amino acids:

30 Boc-Tyr(BrZ)
 Boc-D-Trp
 Boc-D-Trp
 Boc-Tyr(BrZ)

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Example 21

Synthesis of H₂N-Tyr-D-Trp-D-Trp-Phe-Met-CONH₂

The procedure set forth in Example 4 was employed to synthesize the pentapeptide H₂N-Tyr-D-Trp-5 D-Trp-Phe-Met-CONH₂ employing the following sequence of amino acids:

10

Boc-Met
Boc-Phe
Boc-D-Trp
Boc-D-Trp
Boc-Tyr(BrZ)

Example 22

In Vitro Growth Hormone Release Study

Female rats of the CD-1 strain were housed in 15 a constant temperature room at 24°C. with 14 hours light and 10 hours darkness. The rats were fed Purina brand rat chow ab libitum. All studies were started between 0800 and 1000 hours.

Pituitaries were removed from 20 day old 20 female rats. In each of fifteen polytetrafluoroethylene beakers (10 ml) were incubated two pituitaries at 36°C. in 1 ml of lactated Ringer's solution in a Dubnoff Shaker (90 cycles/min.). Three beakers were employed for each of the dosage levels shown in Table 25 II. All medium in each beaker was removed each hour (e.g., P₁, P₂, I₃, I₄) and then fresh medium was added back to each beaker. Each medium removed was assayed for GH, in duplicate, by a standard radioimmunoassay (RIA). The growth hormone radioimmunoassay reagents 30 were distributed by The National Institute of Arthritis and Metabolic Disease Division (NIAMDD) program. The GH values were recorded in terms of nanograms (ng) of a rat standard with a growth hormone potency of 0.61 units/mg.

35

The growth hormone agonist of Example 1 was

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not added to the incubation mediums employed during the first hour of the incubation period (P_1) or to the incubation mediums employed during the second hour of the incubation period (P_2). The growth hormone agonist 5 of Example 1 was dissolved in dimethylsulfoxide (DMSO; 10:1, agonist:DMSO), added to each incubation medium employed during the third hour of the incubation period (I_3) and to each medium employed during the fourth hour of the incubation period (I_4). The release of growth 10 hormone was recorded as ΔGH and calculated by subtracting the amount of GH released at P_2 from that released at I_3 and I_4 . The agonist activity was determined from the release at I_3 and I_4 . The mean of the 6 ΔGH values obtained from each of the three beakers per dosage 15 level measured at I_3 and I_4 are set forth in Table II.

Example 23

In Vitro Growth Hormone Release Study

The procedure set forth in Example 22 was employed in an in vitro growth hormone release study of 20 the peptides of Examples 2-11 and 13-21 and the results therefrom are also set forth in Table II.

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TABLE II
IN VITRO GROWTH HORMONE RELEASE^{1,2}

Peptide	Dosage ³ : Control - 0.3 μg			
	Control	0.01μg	0.1 μg	0.3 μg
H ₂ N-Tyr-D-Trp-Ala-Trp-D-Phe-Gln-COMH ₂	-40±35	240±96 ⁵	2000±523 ⁶	N/A ⁴
H ₂ N-Tyr-D-Trp-Ala-Trp-D-Phe-Thr-COMH ₂	-40±35	94±693 ⁵	650±320 ⁵	N/A
H ₂ N-Tyr-D-Trp-Ala-Trp-D-Phe-Gln-COOH ⁷	-40±35	N/A	753±539 ⁵	N/A
H ₂ N-Tyr-D-Trp-Ala-Trp-D-Phe-COMH ₂	-345±105	N/A	3409±455 ⁵	4540±1200 ⁰
H ₂ N-Tyr-D-Trp-Ser-Trp-D-Phe-COMH ₂	-140±185	N/A	353±144 ⁵	N/A
H ₂ N-Trp-D-Phe-Ala-Tyr-D-Leu-COMH ₂	-96±68	N/A	N/A	N/A
H ₂ N-Trp-D-Phe-Ala-Tyr-Met-COMH ₂	-99±109	N/A	N/A	N/A

1. ΔGH given in terms of ng/ml incubation medium ± standard error of the mean (SEM)
2. P Value <0.001 unless otherwise noted. P value is a comparison of the GH levels in medium containing growth hormone agonist analog to the GH levels in medium (control) without the agonist. Accordingly, the control does not have a P Value.
3. Dosage given in terms of μg/ml incubation medium.
4. N/A denotes not available.
5. P Value not significant.
6. P Value <0.01.
7. P Value ~0.01.
8. P Value <0.05.
9. P Value ~0.02.
10. P Value <0.02.

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TABLE II (Continued)

Peptide	Dosage: 1 μ -10C μ g			100 μ g
	1 μ g	10 μ g	20 μ g	
H ₂ N-Tyr-D-Trp-Ala-Trp-D-Phe-Gln-CO NH ₂	4235±785	4141±576	N/A	N/A
H ₂ N-Tyr-D-Trp-Ala-Trp-D-Phe-Thr-CO NH ₂	2167±591 ⁶	2957±834 ⁶	N/A	N/A
H ₂ N-Tyr-D-Trp-Ala-Trp-D-Phe-Gln-COOH ²	2759±515	3994±1214 ⁶	N/A	N/A
H ₂ N-Tyr-D-Trp-Ala-Trp-D-Phe-CO NH ₂	4611±510	5000±662	N/A	N/A
H ₂ N-Tyr-D-Trp-Ser-Trp-D-Phe-CO NH ₂	3119±413	3831±365	N/A	N/A
H ₂ N-Trp-D-Phe-Ala-Tyr-D-Leu-CO NH ₂	238±67 ⁶	1224±305 ⁷	N/A	1470±487 ⁷
H ₂ N-Trp-D-Phe-Ala-Tyr-Met-CO NH ₂	N/A	1001±207	N/A	882±283 ⁶
H ₂ N-D-Trp-D-Phe-Lys-Met-CO NH ₂	147±88 ⁵	853±138	N/A	2024±254
H ₂ N-Trp-D-Phe-Lys-Met-CO NH ₂	-108±52 ⁵	440±180 ⁸	N/A	861±160 ⁶
H ₂ N-D-Trp-D-Trp-Phe-CO NH ₂	943±137 ⁶	1263±293	N/A	635±165 ⁶
H ₂ N-D-Lys-Tyr-D-Trp-D-Trp-Phe-CO NH ₂	1046±266 ⁶	N/A	1487±197	1654±329
H ₂ N-Tyr-Gly-D-Trp-Phe-D-Phe-CO NH ₂	200±167 ⁵	1384±120	N/A	1196±194 ⁶
H ₂ N-D-Phe-Trp-D-Phe-Phe-Lys-CO NH ₂	-109±101 ⁵	888±120	N/A	963±96 ⁹
H ₂ N-D-Phe-Trp-D-Phe-Phe-Met-CO NH ₂	N/A	1469±153	N/A	1483±168 ⁵
H ₂ N-D-Phe-Phe-D-Trp-Phe-Met-CO NH ₂	130±143 ⁵	955±248	N/A	1060±469 ⁵
H ₂ N-Tyr-D-Trp-D-Trp-Phe-CO NH ₂	686±82 ⁵	1790±60	N/A	984±355 ¹⁰
H ₂ N-Tyr-D-Trp-D-Trp-Tyr-CO NH ₂	1111±572 ⁵	1898±267	N/A	N/A ⁹
H ₂ N-Tyr-D-Trp-D-Trp-Phe-Met-CO NH ₂	1697±222	N/A	1530±208	219±113 ⁹

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TABLE II (Continued)

Peptide	Dosage ³ : Control - 0.3 μ g			
	Control	0.01 μ g	0.1 μ g	0.3 μ g
H ₂ N-D-Trp-D-Phe-D-Phe-Lys-Met-CONH ₂	58±48	N/A	N/A	N/A
H ₂ N-Trp-D-Phe-D-Phe-Lys-Met-CONH ₂	-79±55	N/A	N/A	24±24 ⁵
H ₂ N-D-Trp-D-Trp-Phe-CONH ₂	180±108	N/A	N/A	592±237 ⁹
H ₂ N-D-Iys-Tyr-D-Trp-D-Trp-Phe-CONH ₂	-180±108	N/A	N/A	N/A
H ₂ N-Tyr-Gly-D-Trp-Phe-D-Phe-CONH ₂	-198±85	N/A	N/A	N/A
H ₂ N-D-Phe-Trp-D-Phe-Phe-Lys-CONH ₂	237±264	N/A	N/A	N/A
H ₂ N-D-Phe-Trp-D-Phe-Phe-Met-CONH ₂	146±172	N/A	N/A	N/A
H ₂ N-D-Phe-Trp-D-Phe-Phe-Met-CONH ₂	46±61	N/A	N/A	N/A
H ₂ N-D-Phe-Phe-D-Trp-Phe-Met-CONH ₂	-31±58	N/A	453±48	N/A
H ₂ N-Tyr-D-Trp-D-Trp-Phe-CONH ₂	-170±42	N/A	N/A	345±103 ⁶
H ₂ N-Tyr-D-Trp-D-Trp-Tyr-CONH ₂	-54±35	N/A	N/A	N/A
H ₂ N-Tyr-D-Trp-D-Trp-Phe-Met-CONH ₂				

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The results set forth in Table II demonstrate that peptides within the scope of the instant invention can induce a significant in vitro release of growth hormone from the pituitary.

5 By introducing various other hormones, e.g., somatostatin, testosterone, cortisol, insulin, etc., into the incubation medium of Examples 22 and 23, one can study what effect these latter hormones have on the regulation of growth hormone secretion.

10

Example 24

In Vivo Diagnostic Application

A peptide within the scope of this invention is injected IV into a mammal, including a human. Blood samples are taken before and at +15 minute intervals 15 after the IV injection for about 1 to about 2 hours. Serum growth hormone levels are measured on each of the blood samples. The rise in growth hormone level is an index of the response. The degree of the growth hormone response is indicative of whether the hypothalamic- 20 pituitary unit is functioning normally to secrete growth hormone.

This test can be employed for evaluating whether the hypothalamic-pituitary system is normal under a large number of different clinical and experimental conditions in both healthy and disease states. 25 The test has application at all ages and in both sexes.

Based on this disclosure, many other modifications and ramifications will naturally suggest themselves to those skilled in the art. These are intended 30 to be comprehended as within the scope of this invention.

CLAIMS:

1. A peptide having a formula selected from
5 a group consisting of

- X-Y₁-Z₁-E₁-G₁-J₁-Q
- X-Y₂-Z₂-E₂-G₂-J₂-Q
- X-Y₃-Z₃-E₃-G₃-J₃-Q
- X-Y₄-Z₄-E₄-Q
- 10 X-Y₅-Z₅-E₅-J₅-Q
- X-Y₆-Z₆-E₆-J₆-Q
- X-Y₇-Z₇-E₇-G₇-J₇-Q
- X-Y₈-Z₈-E₈-G₈-Q
- X-Y₉-Z₉-E₉-G₉-J₉-Q
- 15 X'-Y₁₀-Z₁₀-E₁₀-G₁₀-J₁₀-L₁₀-Q

and their pharmaceutically acceptable non-toxic acid addition salts, wherein

X is selected from a group consisting of -NH₂, -NHCH₃, and -N(CH₃)₂;

20 Y₁, G₁, Y₂, G₂, E₄, Z₅, J₅, Y₆, G₆, Z₇, G₇, Y₈, G₈, Y₉, G₉, Y₁₀, and G₁₀ are selected from a group consisting of Tyr, Trp, and Phe;

Z₁, J₁, Z₂, Z₃, E₃, Y₄, Z₄, E₅, G₅, E₆, J₆, Y₇, E₇, Z₈, E₈, Z₉, E₉, Z₁₀, and J₁₀ are selected from
25 a group consisting of D-Tyr, D-Trp, and D-Phe;

J₃ and Z₆ are selected from a group consisting of Gly, Ala, Val, Leu, Ile, Pro, HyPro, Ser, Thr, Cys, and Met;

E₁ is selected from a group consisting of
30 Gly, Ala, Val, Leu, Ile, Pro, HyPro, Ser, Thr, Cys, Met, Asp, Gln, and His;

E₂ is selected from a group consisting of
Gly, Ala, Val, Leu, Met, and Ile;

J₂ is selected from a group consisting of
35 Gly, Ala, D-Ala, Val, D-Val, Leu, D-Leu, Ile, D-Ile,

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Pro, D-Pro, Hypro, D-Hypro, Ser, D-Ser, Thr, D-Thr, Cys, D-Cys, Met, and D-Met;

Y_3 is selected from a group consisting of Tyr, D-Tyr, Trp, D-Trp, Phe, and D-Phe;

5 G_3 is selected from a group consisting of Lys and Arg;

Y_5 is selected from a group consisting of D-Lys and D-Arg;

10 J_7 is selected from a group consisting of Gly, Ala, Val, Leu, Ile, Pro, Hypro, Ser, Thr, Cys, Met, Asp, Glu, Asn, Gln, Arg, and Lys;

J_9 is selected from a group consisting of natural amino acids and the D-configuration thereof;

15 X' is selected from a group consisting of $-NHCOCH_3$, $-NH_2$, $-NHCH_3$, and $-N(CH_3)_2$;

E_{10} is selected from a group consisting of Gly, Ala, Val, Leu, Ile, Ser, Thr, Met, Asp, and Gln;

L_{10} is selected from a group consisting of Asn, Gln, Glu, Arg, Lys, Ser, and Thr; and

20 Q is selected from a group consisting of $-CONH_2$, $-CONHR$, $-CONR_1R_2$, $-CH_2OR$, $-CH_2OH$, $-COOH$, and $-COOR$,

wherein each R, R_1 , and R_2 is selected from a group consisting of straight and branched alkyl groups containing 1-6 carbon atoms.

2. The peptide of claim 1 having the formula selected from the group consisting of

$X-Tyr-D-Trp-E_1-Trp-D-Phe-Q$

$X-Trp-D-Phe-Ala-Tyr-J_2-Q$

30 $X-Y_3-D-Phe-D-Phe-Lys-Met-Q$

$X-D-Trp-Z_4-E_4-Q$

$X-D-Lys-Tyr-D-Trp-D-Trp-Phe-Q$

$X-Tyr-Gly-D-Trp-Phe-D-Phe-Q$

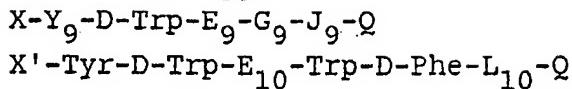
$X-D-Phe-Z_7-E_7-Phe-J_7-Q$

35 $X-Y_8-D-Trp-E_8-G_8-Q$

$X-Tyr-D-Trp-D-Trp-Tyr-Q$

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wherein

E_4 , Z_7 , G_8 , and G_9 are selected from the
5 group consisting of Trp and Phe;

Z_4 , E_7 , E_8 , and E_9 are selected from the
group consisting of D-Trp and D-Phe;

E_1 is selected from the group consisting of
Gly, Ala, Val, Leu, Ile, Ser, Thr, Asn, and Gln;

10 J_2 is selected from the group consisting of
DLeu and Met;

Y_3 is selected from the group consisting of
Trp and D-Trp;

J_7 is selected from a group consisting of Lys
15 and Met;

J_9 is selected from a group consisting of
Met, D-Met, Leu, D-Leu, Phe, D-Phe, Arg, D-Arg, Pro,
and D-Pro;

20 E_{10} is selected from the group consisting of
Gly, Ala, Val, Leu, Ile, Ser, Thr, Asn, and Gln; and

L_{10} is selected from the group consisting of
Asn, Gln, Arg, Lys, Ser, and Thr.

3. The peptide of claim 2 having the
formula

25 $X-Tyr-D-Trp-Ala-D-Phe-L_{10}-Q$.

4. The peptide of claim 1 having a formula
selected from the group consisting of

$X-Tyr-D-Trp-Ala-D-Phe-Gln-Q$; and

$X-Tyr-D-Trp-Ala-D-Phe-Thr-Q$.

30 5. The peptide of claim 1 having a formula
selected from a group consisting of

$H_2N-Tyr-D-Trp-Ala-Trp-D-Phe-CONH_2$;

$H_2N-Tyr-D-Trp-Ser-Trp-D-Phe-CONH_2$;

$H_2N-Tyr-D-Trp-Ala-Trp-D-Phe-COOH$;

35 $H_2N-Trp-D-Phe-Ala-Tyr-D-Leu-CONH_2$;

$H_2N-Trp-D-Phe-Ala-Tyr-Met-CONH_2$;

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$\text{H}_2\text{N-D-Trp-D-Phe-D-Phe-Lys-Met-CONH}_2$;
 $\text{H}_2\text{N-Trp-D-Phe-D-Phe-Lys-Met-CONH}_2$;
 $\text{H}_2\text{N-D-Trp-D-Trp-Phe-CONH}_2$;
 $\text{H}_2\text{N-D-Lys-Tyr-D-Trp-D-Trp-Phe-CONH}_2$;
5 $\text{H}_2\text{N-Tyr-Gly-D-Trp-Phe-D-Phe-CONH}_2$;
 $\text{H}_2\text{N-D-Phe-Trp-D-Phe-Phe-Lys-CONH}_2$;
 $\text{H}_2\text{N-D-Phe-Trp-D-Phe-Phe-Met-CONH}_2$;
 $\text{H}_2\text{N-D-Phe-Phe-D-Trp-Phe-Met-CONH}_2$;
 $\text{H}_2\text{N-Tyr-D-Trp-D-Trp-Tyr-CONH}_2$;
10 $\text{H}_2\text{N-Tyr-D-Trp-D-Trp-Phe-Met-CONH}_2$;
 $\text{H}_2\text{N-Tyr-D-Trp-Ala-Trp-D-Phe-Gln-CONH}_2$;
 $\text{H}_2\text{N-Tyr-D-Trp-Ser-Trp-D-Phe-Thr-CONH}_2$;
 and
 $\text{H}_2\text{N-Tyr-D-Trp-Ala-Trp-D-Phe-Gln-COOH}$.

15 6. The peptide of claims 1-3 or 4 wherein each R, R_1 , and R_2 of Q is selected from the group consisting of alkyl groups containing 1-2 carbon atoms.

7. The peptide of claims 1-3 or 4 wherein Q is -CONH_2 .

20 8. A method of releasing growth hormone from a pituitary comprising contacting said pituitary with the peptide of claims 1-6 or 7.

9. A compound of a formula selected from a group consisting of

25 Boc-Tyr(BrZ)-D-Trp-Ala-Trp-D-Phe-@;
 Boc-Tyr(BrZ)-D-Trp-Ala-Trp-D-Phe-@';
 Boc-Tyr(BrZ)-D-Trp-Ser(Bz)-Trp-D-Phe-@;
 Boc-Trp-D-Phe-Ala-Tyr(BrZ)-Met-@;
 Boc-Trp-D-Phe-Ala-Tyr(BrZ)-D-Leu-@;
 Boc-D-Trp-D-Phe-D-Phe-Lys(Z)-Met-@;
 Boc-Trp-D-Phe-D-Phe-Lys(Z)-Met-@;
 Boc-D-Trp-D-Trp-Phe-@;
 Boc-D-Lys(Z)-Tyr(BrZ)-D-Trp-D-Trp-Phe-@;
 Boc-Tyr(BrZ)-Gly-D-Trp-Phe-D-Phe-@;
 Boc-D-Phe-Trp-D-Phe-Phe-Lys(Z)-@;
 Boc-D-Phe-Trp-D-Phe-Phe-Met-@;

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Boc-D-Phe-Phe-D-Trp-Phe-Met-@;
Boc-Tyr(BrZ)-D-Trp-D-Trp-Phe-@;
Boc-Tyr(BrZ)-D-Trp-D-Trp-Tyr(Z)-@;
Boc-Tyr(BrZ)-D-Trp-D-Trp-Phe-Met-@;
5 Boc-Tyr(BrZ)-D-Trp-Ala-Trp-D-Phe-Gln-@;
Boc-Tyr(BrZ)-D-Trp-Ala-Trp-D-Phe-Gln-@';
and
Boc-Tyr(BrZ)-D-Trp-Ala-Trp-D-Phe-Thr-@

wherein

10 BrZ is p-bromobenzylloxycarbonyl;
Boc is t-butoxycarbonyl;
Bz is benzyl;
Z is benzyloxycarbonyl;
@' is hydroxymethyl resin; and
15 @ is benzhydrylamine resin.



European Patent
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PARTIAL EUROPEAN SEARCH REPORT
which under Rule 45 of the European Patent Convention
shall be considered, for the purposes of subsequent
proceedings, as the European search report

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EP 80 30 0700

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int. Cl.)
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X	FR - A - 2 347 336 (ICI) * Entire document * --	1-5,7	
X	DE - A - 2 739 440 (COY, D.H.) * Entire document * --	1-3,5, 7	
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	BIOCHEMICAL AND BIOPHYSICAL RE- SEARCH COMMUNICATION, vol. 73, no.	1	
INCOMPLETE SEARCH			CATEGORY OF CITED DOCUMENTS
<p>The Search Division considers that the present European patent application does not comply with the provisions of the European Patent Convention to such an extent that it is not possible to carry out a meaningful search into the state of the art on the basis of some of the claims.</p> <p>Claims searched completely: 1-7,9</p> <p>Claims searched incompletely:</p> <p>Claims not searched: 8</p> <p>Reason for the limitation of the search: Method for treatment of the human or animal body by surgery or therapy (See article 52(4) of the European Patent Convention)</p>			X: particularly relevant A: technological background O: non-written disclosure P: Intermediate document T: theory or principle underlying the invention E: conflicting application D: document cited in the application L: citation for other reasons &: member of the same patent family, corresponding document
Place of search	Date of completion of the search	Examiner	
La Haye	10-07-1980	RAJIC	



European Patent
Office

PARTIAL EUROPEAN SEARCH REPORT

0018072
EP 86300700
Application number

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Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.)
			TECHNICAL FIELDS SEARCHED (Int. Cl.)
	<p>3, 1976, Academic Press, Inc. D.H. COY et al. "Synthesis and opioid activities of stereoisomers and other D-amino acid analogs of methionine-enkephalin". pages 632-38.</p> <p>* Entire document**</p> <p>--</p> <p>JOURNAL OF THE CHEMICAL SOCIETY, 1965, Part V, The Chemical Society, London, GB D.S. JONES et al. "Peptides. Part XVII. Synthesis of Peptides and Polymers of Some Sterically Hindered Amino-Acids via Oxazolone Intermediates", pages 6227-39.</p> <p>* Entire article *</p> <p>-----</p>	1	

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